

DEVELOPMENT OF “IN VITRO” MODELS TO STUDY WOUND HEALING IN SPACE THE SUTURE EXPERIMENT

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Providing adequate medical care in space will become challenging in the future exploration missions.

Trauma care and surgical emergency in space missions

The feasibility of a number of procedures and equipments for trauma care and basic surgery in space has been evaluated.

The International Space Station (ISS) is equipped with an Advanced Life Support Pack able to deliver Advanced Cardiac Life Support (ACLS) Advanced Trauma Life Support (ATLS).

However, the current management of traumatic events and surgical emergency requires patient stabilization and rapid return to Earth.

Comprehensive methodologies to guide the crew members remotely have been developed, but they are appropriate for low Earth orbit and lunar missions (< 5sec delay in communication)

Problems of medical care in future space exploration missions



In interplanetary missions medical evacuation times to Earth might become too long.

The communication lag would render useless to guide the crew actions remotely.

Therefore, the future planning procedure for medical care in space should incorporate space surgery and trauma care concepts. Critical aspects in surviving a trauma or a surgery are wound/suture behavior and healing.

Background

A recent report on the “state of the art” in space surgery included wound healing and sutures among the critical aspect which have been poorly studied and need to be further investigated.

The relatively little literature on wound healing in weightlessness is controversial.

The prevailing evidence and our previous studies indicate an impairment of the healing process.

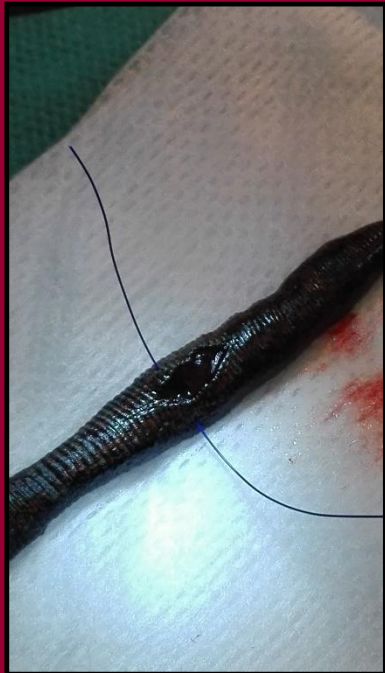
It could be due to homeostatic alterations occurring in microgravity (μg) and/or changes in some aspects of the biological response to wounds and lesions, such as hemorrhage progression and bacterial flora behavior.

Preliminary reports on animals do not offer any definite conclusions.

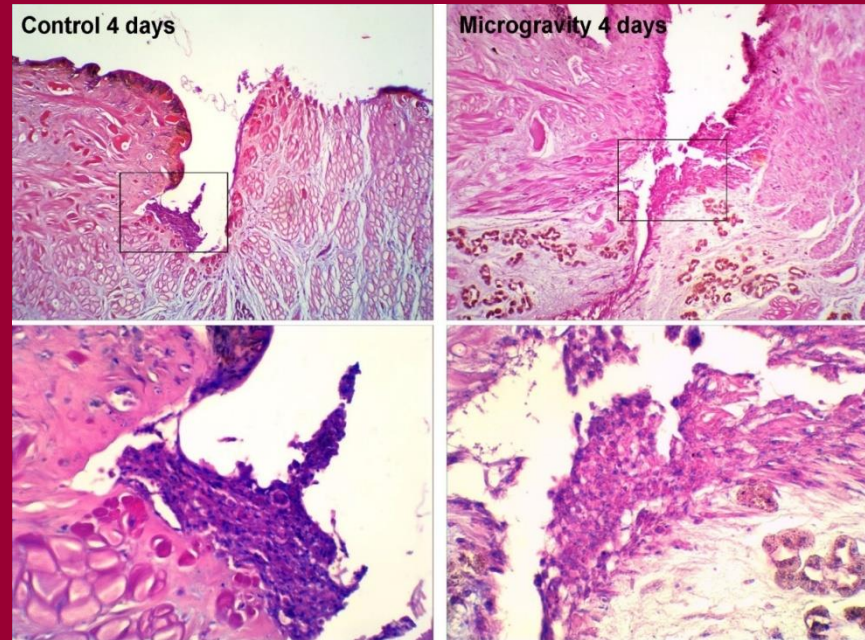
Wound Healing in μg : the *Hirudo medicinalis* model

This invertebrate is an interesting model for wound healing studies since the sequence of events occurring in this process is the same as in vertebrates.

A surgical lesion (≈ 1 cm) is performed on the dorsal part of the animal. The two edges of the wound can be sutured.



Model preparation



Histology of the wound after 4 days of exposure to μg .

In vitro studies on processes involved in wound healing

Results show alteration of repair mechanisms: abnormal cell migration and collagen formation, increased inflammation and decreased cellular organization

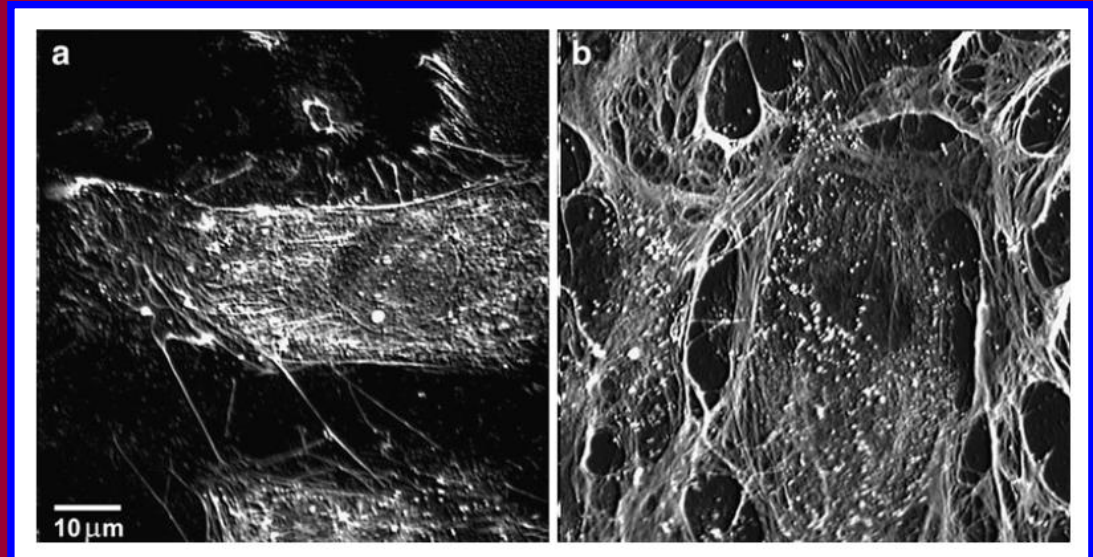
Our previous studies [Morbidelli et al., 2005; Monici et al., 2006], demonstrated that μg alters extracellular matrix (ECM) and endothelial function, thus possibly affecting edema behavior and tissue stiffness.

Recently, we observed that fibroblast function [Monici et al., 2017] and endothelial-stromal interaction are impaired.

Fibronectin expression in endothelial cells assessed by immunofluorescence:

a) 1xg control

b) cells exposed for 3 days to μg modeled by a RPM



WOUND HEALING AND SUTURES IN UNLOADING CONDITIONS

It is an experiment selected by ESA (ILSRA-2014-0043) to be performed on the ISS. It is conceived to study in weightlessness the behavior and healing of “in vitro” sutured wound models.

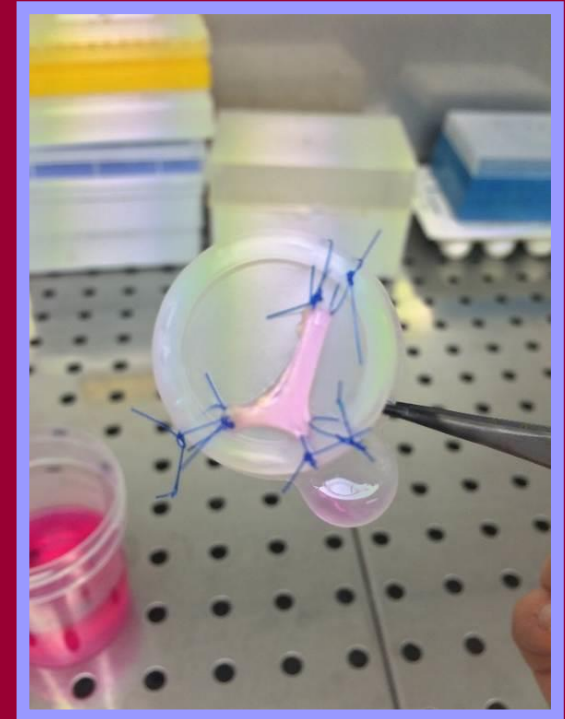
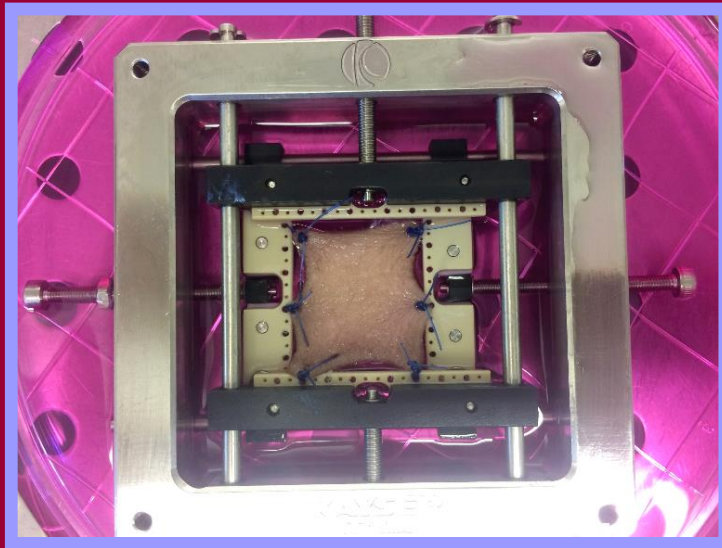
General Objectives

- know the behaviour of sutures in μg , in terms of ability of the wound margins to merge, restore the tensile strength in the tissues around the wound and favor healing. In microgravity, a different impact of the suture in terms of mechanical stress induced in the tissue may be expected.
- Get information on evolution of the healing and scar quality in μg
- Understand how suturing materials and techniques can be adapted to the μg environment, thus developing strategies to manage wounds in space and promote healing.

IN VITRO MODEL OF SUTURED WOUND (I)

Models are based on skin and vein vessel biopsies collected, after informed consent*, from healthy patients undergoing abdominoplasty surgery, hernia repair and breast reduction.

After collection, the tissue biopsies are fixed to a frame specifically developed to apply a tensile strength similar to the physiological one (~ 10 N) and to measure tension changes.



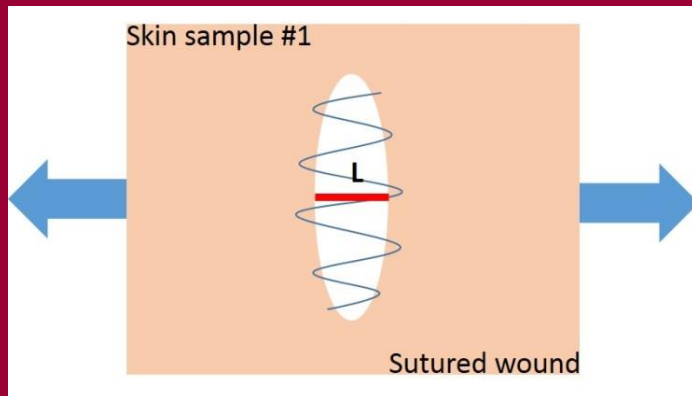
The samples are maintained in culture medium (RPMI) at 4°C for storage and travelling to the launch site.

*Following the rules of our Institution we do not need the approval of the Ethic Committee because we obtain the biopsies from abdominoplasty surgery, breast reduction, hernia repair. This material is normally thrown away and the use of it does not interfere with the patient, the therapies administered to the patient or the procedures for surgery.

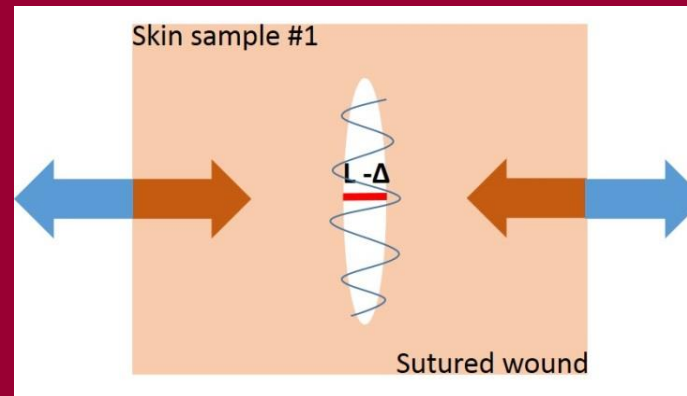
IN VITRO MODEL OF SUTURED WOUND (II)

At the launch site, linear wounds (10 mm length, 2 mm depth) are performed on skin by a scalpel and then sutured with interrupted stitch 3.0-4.0 non absorbable suture (Nylon). The vessels are completely divided to perform an end-to-end vascular anastomosis with continuous 6.0-8.0 non absorbable suture (Polypropilene).

The 8 samples (4 skin sutures and 4 vessel sutures) are placed into EUs, filled with culture medium (DMEM). The EUs are placed in ECs and are ready for handover, upload and launch.



T0:
Beginning of the experiment: specimen loaded into the Experiment Unit (EU)
Wound width= L



T1:
Experiment in progress
Wound width= $L - \Delta$

Blue arrow

Tension applied by means of the EU to the specimen to reproduce physiological tension

Orange arrow

Tension produced by the tissue due to the healing process

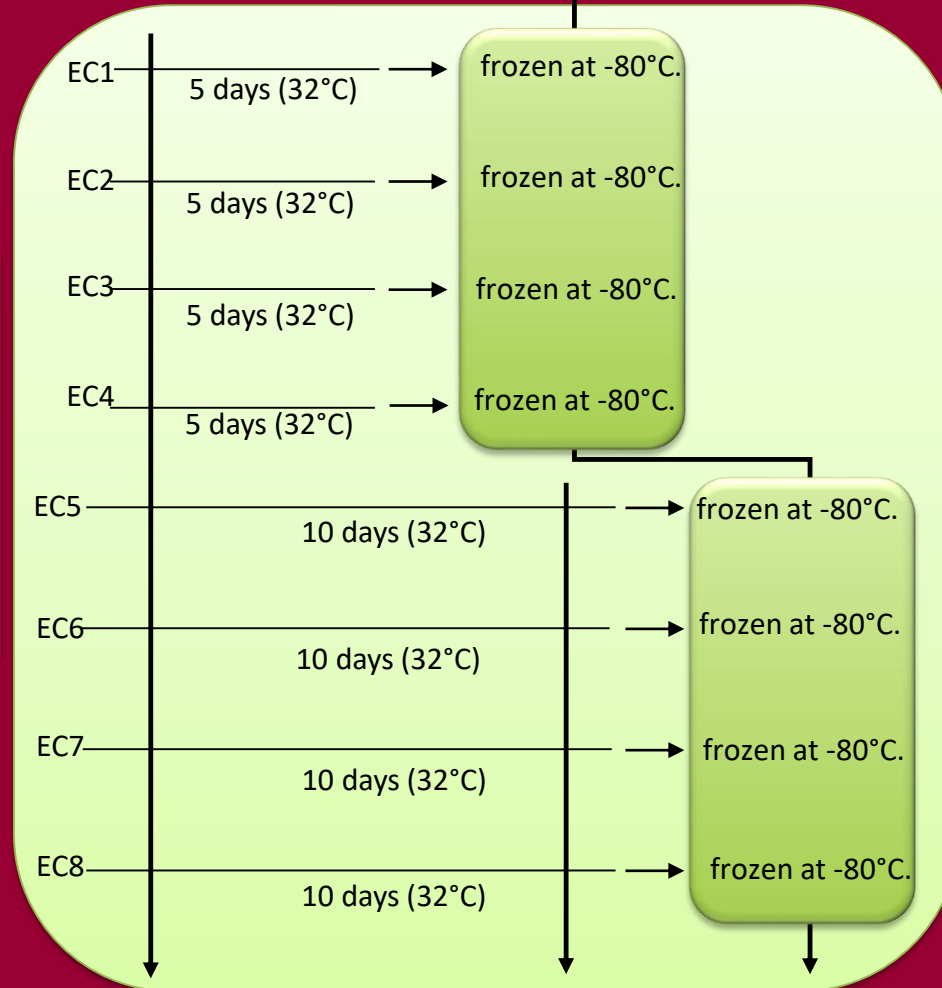
EXPERIMENT FLOW DIAGRAM

Prelaunch

The sutured wound models (4 skin sutures and 4 vessel sutures) will be prepared and placed in Eus with organ culture medium and maintained at about 32°C

Temperature logging
Imaging of the samples

In flight



Temperature logging
Automated imaging
Monitoring of tensile strength in suture models

Temperature logging

Postflight retrieval

Samples to be returned to PI after return to Earth

Preferred T:
≤ -20°C

Temperature logging
Imaging of the samples

Parameters measured inflight

- temperature logging;
- timing of the experiment steps (automated);
- tensile strength in tissue samples;
- morphological imaging of the sutures and wounds.

Parameters measured postflight

- suture morphology and ultrastructure (histology and electron microscopy);
- blood vessels and endothelial function in the sutured tissues;
- proteomics on membrane microdomains;
- gene expression profile in sutured tissues;
- extracellular matrix turnover in sutured tissues;
- apoptosis and necrosis in sutured tissues;
- markers of activation of repair mechanisms and fibroblast behavior in sutured tissues;
- stiffness and strength of small specimens from sutured wounds.

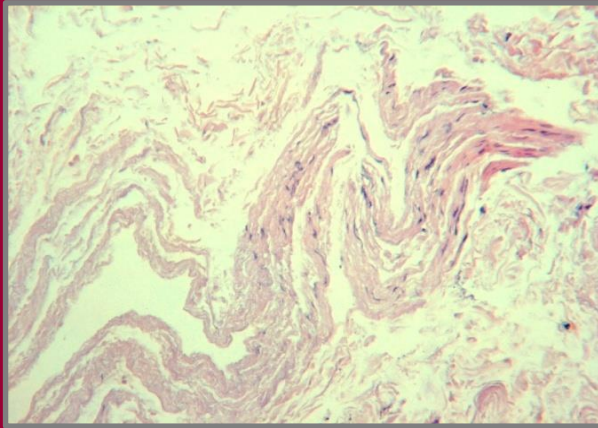
ON GROUND ACTIVITY

- Ensure tissue survival throughout the experiment (3 weeks) improving tissue culture techniques
- Standardize procedures for collecting biopsies, model preparation and culture technique, postflight analysis of samples
- Analyze the mechanical properties of tissues
- Develop a device to model physiological tensile strength in the tissue and measure eventual changes in tensile strength

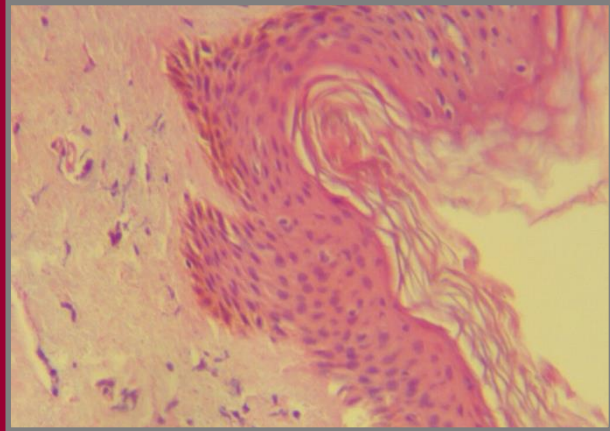
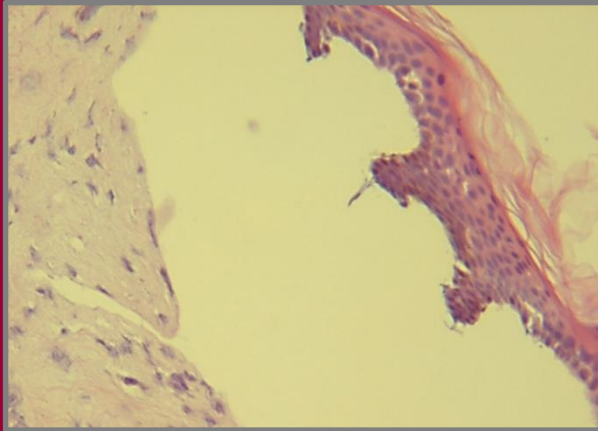
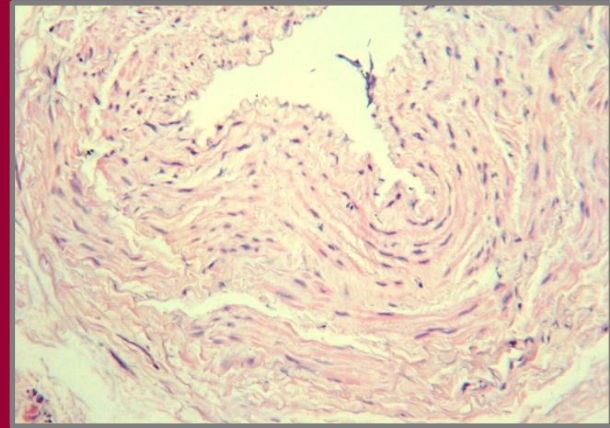
LESSONS LEARNT

Tissue preservation

Unsatisfactory

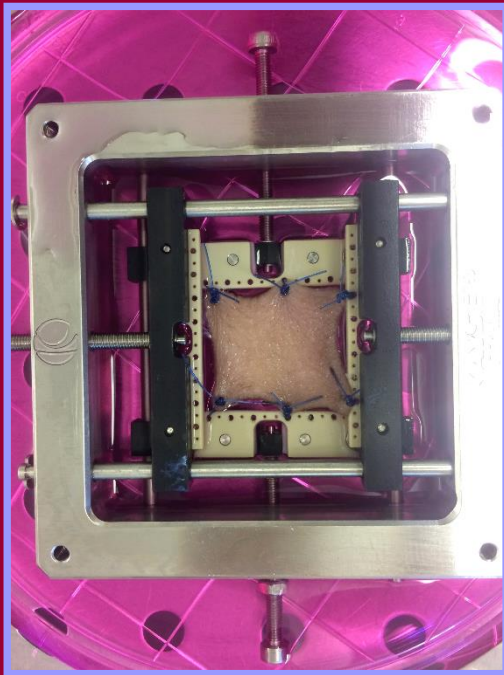


Satisfactory

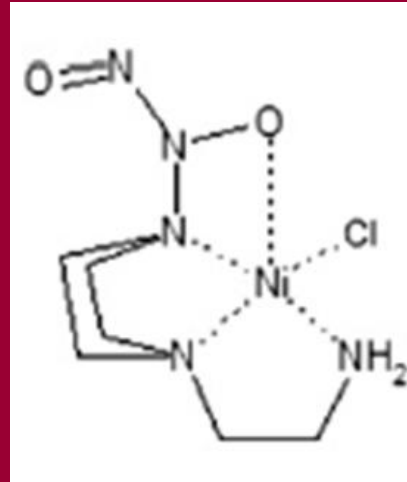


RESULTS

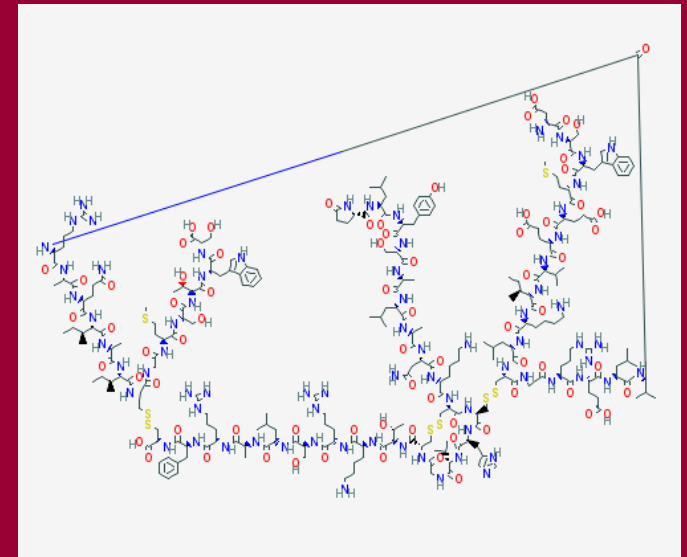
Applying a proper tension and adding hormones (relaxin), pro-angiogenic (metallononoates) and antioxidant factors to culture media, we strongly improved tissue survival as shown by histology.



Experimental device for the application of tensile strength



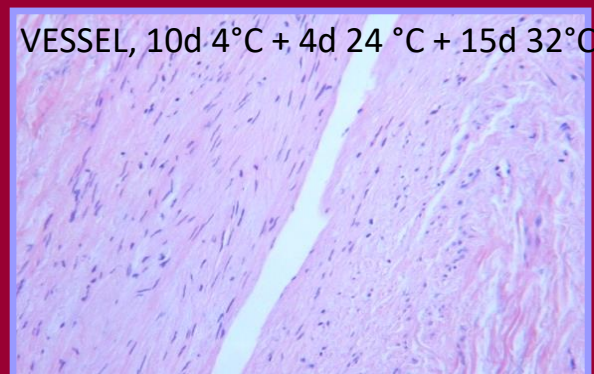
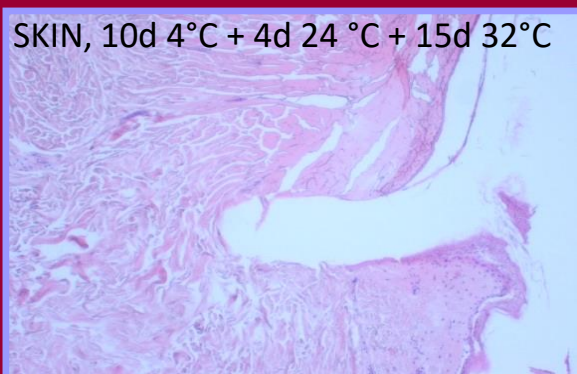
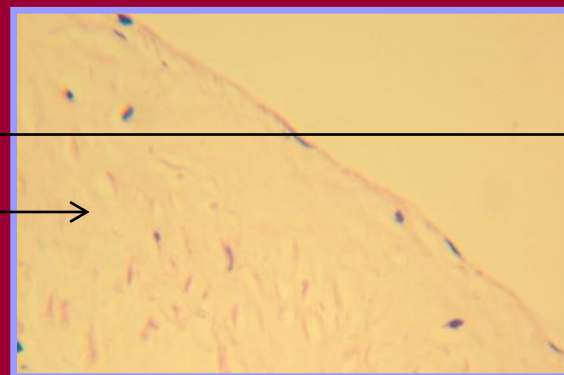
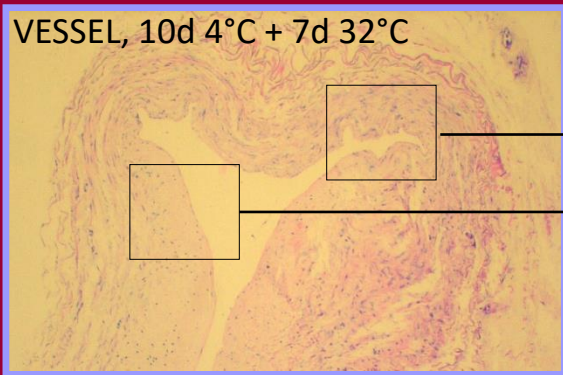
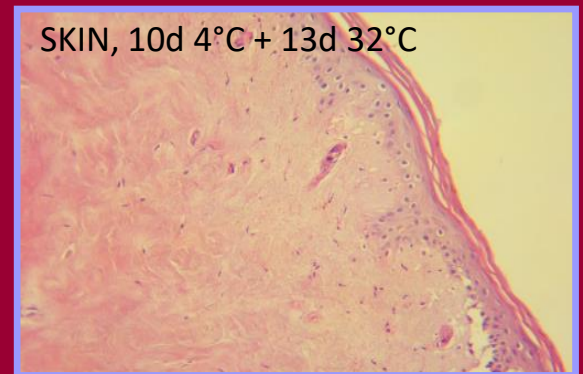
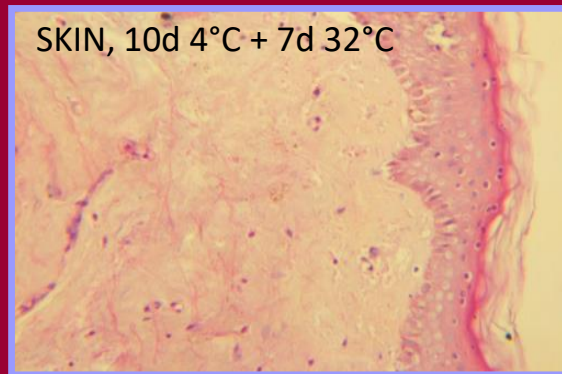
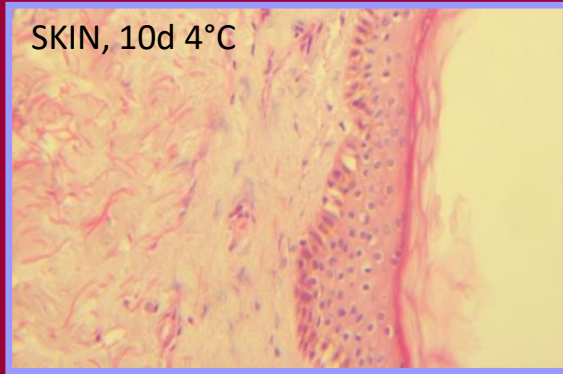
[Ni(PipNONO)Cl]



Relaxin



RESULTS



Histology of skin (left) and vessel samples (right) after 29 days of culture.

CONCLUSIONS

We developed a device and a tissue culture technique able to model tensional strength in tissues and ensure tissue survival for over 3 weeks.

This technique and resulting models can be applied also on ground to:

1. tissue culture and engineering for transplantation and regeneration
2. Studies on tissue mechanical properties
3. Studies for improvement of surgical techniques and materials.

WOUND HEALING AND SUTURES IN UNLOADING CONDITIONS

ILSRA-2014-0043

Scientific team coordinator

Monica Monici

Partners

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<i>Marcel Egli</i>	Space Biology	<i>Paolo Romagnoli</i>	Histology
<i>Daniela Grimm</i>	Cell Biology	<i>Theodoor Smit</i>	Mechanobiology
<i>Jack van Loon</i>	Space Biology		



**Thank you for
attention**

EXPECTED RESULTS

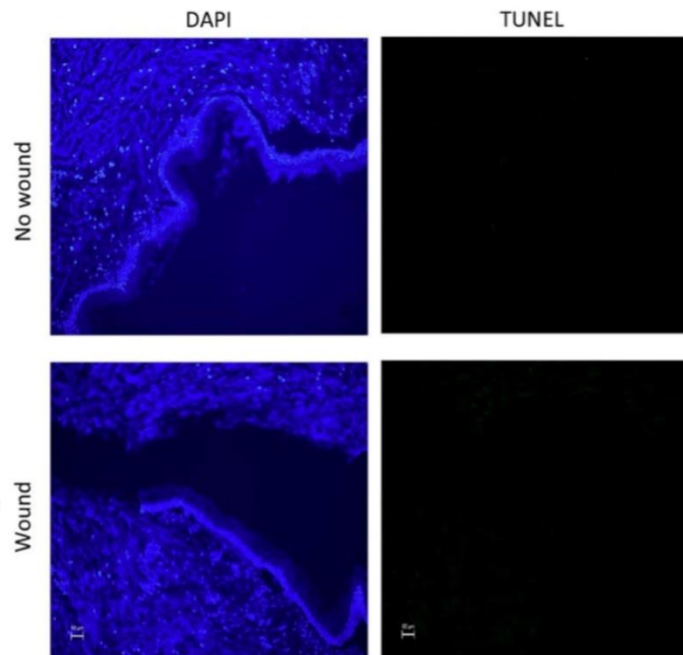
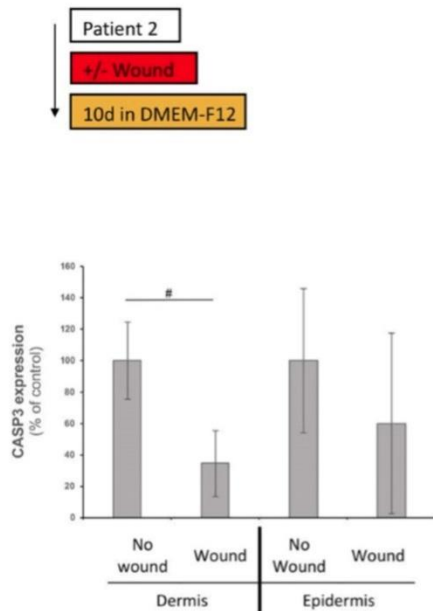
- Increase knowledge on the molecular, cellular and tissue mechanisms involved in the healing of sutured wounds.
- Gain information about the role of mechanical stress, loading and unloading conditions in tissue repair.
- Support the development of strategies to promote tissue repair in unloading conditions and also on Earth.
- Transfer of knowledge to solve healing problems in frail subjects on Earth, e.g., elderly, diabetic and/or bedridden patients.
- Support to long-duration human space exploration as regards health issues: how to manage accidental injuries due to traumatic events and emergency surgery on board of Space Stations and Space Vehicles.

METHODS

(hydrocortison, insulin and ascorbic acid) and antibiotics (penicillin, gentamycin, amphotericin B).

Histology (skin)

Samples were fixed in 4% formaldehyde in 0.1 M phosphate-buffer (pH 7.4), dehydrated in graded ethanol and embedded in paraffin for light microscopy. Histological sections, 6 μ m thick, stained with hematoxylin & eosin were examined and photographed under a Reichert Microstar IV light microscope equipped with an Eureka 9 high-resolution videocamera (BEL Engineering, Monza, Italy) interfaced with a PC by a dedicated software (BELview, BEL Engineering). As it can be observed, histological analysis shows that the tissue is well preserved



Apoptosis assays

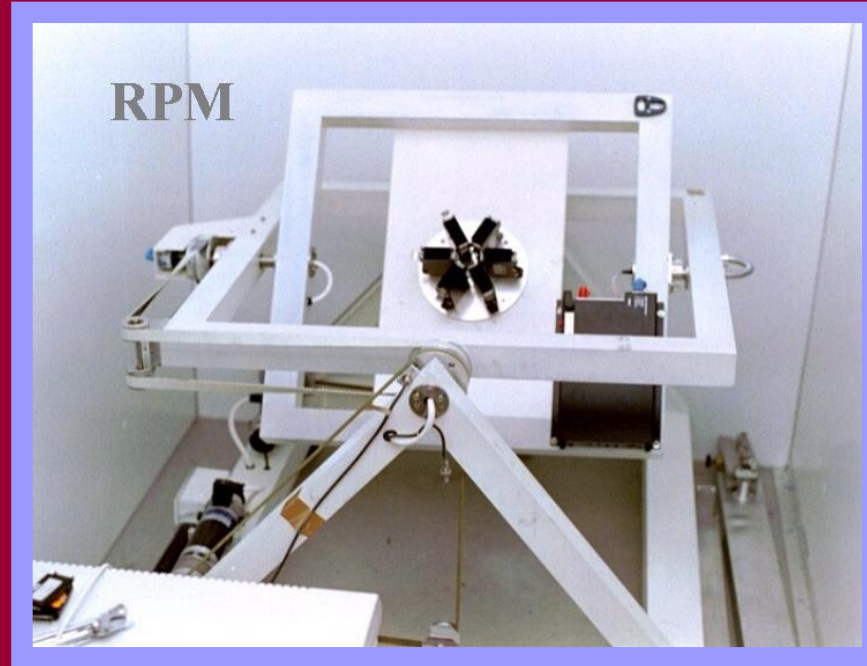
Caspase 3 (apoptosis marker) expression does not increase in wounded samples.

Tunel reaction is based on labeling of DNA strand breaks (TUNEL technology) by a fluorescent probe.

As the pictures show, the Tunel assay is negative (the tissues appear dark and the fluorescent signal is not detectable).

In conclusion, in the tissue cultures analysed, we did not find a significant increase in apoptosis.

Vector averaged gravity



Hypogravity conditions were modelled by a Random Positioning Machine (RPM) developed by Fokker Space, Leiden, The Netherlands. Angular velocity $60^\circ/\text{sec.}$, temperature 37°C .